(FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS, MEDICONF' ENTERED AT 17:45:50 ON 29 OCT 2002)

DEL HIS

L1 27606 S RETROVIR? VECTOR?
L2 348 S L1 AND INTRON
L3 86 S L2 AND SPLICE
L4 31 DUP REM L3 (54 DUPLICATES REMOVED)
L5 31 SORT L4 PY
L6 21 S L5 AND (DONOR OR ACCEPTOR OR SD OR SA)
L7 21 FOCUS L6 1-
L8 10 S L4 AND PY<=1996
L9 10 SORT L8 PY
L10 386 S L1 AND (SPLICE DONOR OR SPLICE ACCEPTOR OR SD OR SA)
L11 91 S L10 AND (RRE OR REV? OR TAT?)
L12 24 S L11 AND INTRON
L13 11 DUP REM L12 (13 DUPLICATES REMOVED)
L14 11 SORT L13 PY

=> d an ti so au ab pi l14 2 3 4 5 6 8

L14 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2002 ACS
AN 1998:268655 CAPLUS
DN 128:291139
TI Construction of TRIN **retroviral vectors** contg. **Rev**-responsive element of HIV1 virus
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
IN Kingsman, Susan Mary; Kingsman, Alan John
AB **Retroviral vector** particles having an RNA genome carrying sequences which provide in the DNA provirus at least one selected gene located within an **intron** in a transcription unit of the provirus, which transcription unit further comprises a polynucleotide response element which is responsive to a nucleus to cytoplasm transport factor such as HIV **Rev**. These vectors have been named TRIN (**Tat** and **Rev** inducible) vectors. Expression of the selected genes is thus rendered **Rev**-dependent and so is dependent upon the presence of HIV. The TRIN vectors also contain the murine leukemia virus **splice donor** site, the strong CMV promoter, a packaging signal, and the HIV U5 and R regions.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9817817	A1	19980430	WO 1997-GB2859	19971017
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9747124	A1	19980515	AU 1997-47124	19971017
GB 2331989	A1	19990609	GB 1999-4143	19971017
GB 2331989	B2	20000927		
EP 931157	A1	19990728	EP 1997-909438	19971017
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001502904	T2	20010306	JP 1998-519088	19971017
US 2002141978	A1	20021003	US 1999-254529	19990804

L14 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2002 ACS
AN 1999:223067 CAPLUS
DN 130:247864
TI **Retroviral vectors** for hematopoietic stem cell transformation comprising functional **splice donor** and acceptor sites formed by **reverse** transcription of a pro-vector
SO PCT Int. Appl., 279 pp.
CODEN: PIXXD2
IN Lewis, Claire; Binley, Katie Mary; Bebbington, Chris; Naylor, Stuart
AB A **retroviral vector** is claimed comprising a functional

splice donor site and a functional **splice acceptor** site; wherein the functional **splice donor** site and the functional **splice acceptor** site flank a first nucleotide sequence of interest ("NOI"); wherein the functional **splice donor** site is upstream of the functional **splice acceptor** site; wherein the **retroviral vector** is derived from a retroviral pro-vector; wherein the retroviral pro-vector comprises a first nucleotide sequence ("NS") capable of yielding the functional **splice donor** site and a second NS capable of yielding the functional **splice acceptor** site; wherein the first NS is downstream of the second NS; such that the **retroviral vector** is formed as a result of **reverse** transcription of the retroviral pro-vector. This system for generating **retroviral vectors** is useful for transfection and gene therapy offers improved safety. Use of this method with specific vectors for the transformation of hematopoietic stem cells is described.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9915694	A2	19990401	WO 1998-GB2885	19980923
	WO 9915694	A3	19990510		
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2303663	AA	19990401	CA 1998-2303663	19980923
	AU 9893562	A1	19990412	AU 1998-93562	19980923
	AU 747609	B2	20020516		
	GB 2345063	A1	20000628	GB 2000-6993	19980923
	GB 2345063	B2	20020724		
	EP 1017838	A2	20000712	EP 1998-946556	19980923
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2001517453	T2	20011009	JP 2000-512973	19980923
	WO 2000017371	A1	20000330	WO 1999-GB3181	19990922
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9962130	A1	20000410	AU 1999-62130	19990922
	EP 1115877	A1	20010718	EP 1999-949142	19990922
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002526083	T2	20020820	JP 2000-574270	19990922
	NO 2000001487	A	20000523	NO 2000-1487	20000322

L14 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS
AN 1999:223066 CAPLUS
DN 130:247863
TI **Retroviral vectors** comprising functional **splice donor** and acceptor sites formed by **reverse** transcription of a pro-vector
SO PCT Int. Appl., 138 pp.
CODEN: PIXXD2
IN Bebbington, Chris; Kingsman, Susan; Uden, Mark; Kingsman, Alan; Mitrophanos, Kyriacos
AB A **retroviral vector** is claimed comprising a functional **splice donor** site and a functional **splice acceptor** site; wherein the functional **splice donor** site and the functional **splice acceptor** site flank a first nucleotide sequence of interest ("NOI"); wherein the

functional **splice donor** site is upstream of the functional **splice acceptor** site; wherein the **retroviral vector** is derived from a retroviral pro-vector; wherein the retroviral pro-vector comprises a first nucleotide sequence ("NS") capable of yielding the functional **splice donor** site and a second NS capable of yielding the functional **splice acceptor** site; wherein the first NS is downstream of the second NS; such that the **retroviral vector** is formed as a result of **reverse** transcription of the retroviral pro-vector. This system for generating **retroviral vectors** is useful for transfection and gene therapy offers improved safety.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9919683	A1	19990401	WO 1998-GB2867	19980923
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2304259	AA	19990401	CA 1998-2304259	19980923
	AU 9891756	A1	19990412	AU 1998-91756	19980923
	AU 750110	B2	20020711		
	GB 2344592	A1	20000614	GB 2000-6992	19980923
	EP 1017837	A1	20000712	EP 1998-944085	19980923
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2001517452	T2	20011009	JP 2000-512972	19980923
	NO 2000001486	A	20000525	NO 2000-1486	20000322

L14 ANSWER 5 OF 11 MEDLINE
AN 2001022046 MEDLINE
TI Split-**intron retroviral vectors**: enhanced expression with improved safety.
SO JOURNAL OF VIROLOGY, (2000 Mar) 74 (5) 2365-71.
Journal code: 0113724. ISSN: 0022-538X.
AU Ismail S I; Kingsman S M; Kingsman A J; Uden M
AB The inclusion of retrovirus-derived **introns** within retrovirus-based expression vectors leads to a fraction of the resulting transcripts being spliced. Such splicing has been shown to markedly improve expression (W. J. Krall et al., Gene Ther. 3:37-48, 1996). One way to improve upon this still further might involve the use of more efficient **introns** instead of those from the provirus. Currently, however, incorporation of such **introns** remains self-defeating since they are removed in the nucleus of the producer cell. In the past, elaborate ways to overcome this problem have included the use of alphaviruses to make the vector transcripts within the cytoplasm, thus avoiding the nuclear splicing machinery during vector production (K. J. Li and H. Garoff, Proc. Natl. Acad. Sci. USA 95:3650-3654, 1998). We now present a novel design for the inclusion of **introns** within a **retroviral vector**. In essence, this is achieved by exploiting the retroviral replication process to copy not only the U3 promoter but also a synthetic **splice donor** to the 5'-long-terminal-repeat position during **reverse** transcription. Once copied, synthesized transcripts then contain a **splice donor** at their 5' end capable of interacting with a consensus **splice acceptor** engineered downstream of the packaging signal Upon transduction, we demonstrate these vectors to produce enhanced expression from near fully spliced (and thus packaging signal minus) transcripts. The unique design of these high titer and high-expression **retroviral vectors** may be of use in a number of gene therapy applications.

L14 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS
AN 2000:688391 CAPLUS
DN 133:262286
TI **Retroviral vectors** comprising functional and

non-functional **splice donor** and **splice acceptor** sites

SO PCT Int. Appl., 148 pp.
CODEN: PIXXD2

IN Uden, Mark; Mitrophanous, Kyriacos

AB **Retroviral vectors** are provided comprising a functional **splice donor** site (FSDS) and a functional **splice acceptor** (FSAS) site, wherein the FSDS and the FSAS flank a first nucleotide sequence of interest (NOI) and the FSDS is upstream of the FSAS.. The **retroviral vector** is derived from a retroviral pro-vector, such that the retroviral pro-vector comprises a first nucleotide sequence (NS) capable of yielding the functional **splice donor** site (FSDS), a second NS capable of yielding the functional **splice acceptor** site (FSAS), a third NS capable of yielding a non-functional **splice donor** site (NFSDS), and a fourth NS capable of yielding a non-functional splice site (NFSS). The first NS is downstream of the second NS and the third NS and fourth NS are upstream of the second NS, such that after **reverse** transcription of the retroviral pro-vector at a desired target site the **retroviral vector** is capable of being spliced. In addn., hybrid adenolentiviral systems are provided, comprising single or multiple adenoviral primary vectors which encodes or encode a retroviral secondary vector. Murine leukemia and equine infectious anemia virus vectors (pICUT) contain a strong **splice acceptor** upstream of the **splice donor** and therefore no functional **intron**; thus, when the vector is transfected into producer cells the resulting transcripts generated will not be spliced and the packaging signal will not be lost and maximal packaging is achievable. Upon transduction, transcripts generated from integrated pICUT vectors contain a strong **splice donor** 5'- of a strong **splice acceptor**, both of which being located upstream of the neo ORF, and therefore contain a functional **intron** in the 5'UTR and thus are maximally spliced and translated. Because the **intron** is created only upon transduction it is possible to limit gene expression to either packaged or transduced cells.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000056910	A1	20000928	WO 2000-GB1091	20000322
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
GB 2362886	A1	20011205	GB 2001-20709	20000322
EP 1163356	A1	20011219	EP 2000-911135	20000322
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

L14 ANSWER 8 OF 11 MEDLINE

AN 2001149475 MEDLINE

TI Human immunodeficiency virus type 2 lentiviral vectors: packaging signal and **splice donor** in expression and encapsidation.

SO JOURNAL OF GENERAL VIROLOGY, (2001 Feb) 82 (Pt 2) 425-34.
Journal code: 0077340. ISSN 0022-1317.

AU D'Costa J; Brown H; Kundra P; Davis-Warren A; Arya S

AB **Retroviral vectors** provide the means for gene transfer with long-term expression. The lentivirus subgroup of retroviruses, such as human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), possesses a number of regulatory and accessory genes and other special elements. These features can be exploited to design vectors for transducing non-dividing as well as dividing cells with the potential for regulated transgene expression. Encapsidation of the transgene RNA in lentiviral vectors is determined by the leader sequence-based multipartite packaging signal. Embedded in the packaging signal is a major **splice donor** site that, this study shows, is not by

itself essential for transgene expression or encapsidation. We designed HIV-2 vectors that contained all the sequence elements thought to be necessary and sufficient for vector RNA encapsidation. Unexpectedly, despite abundant expression, only a small fraction of the transgene RNA was encapsidated and the titre of the vector was low. Redesign of the vector with a mutant **splice donor** resulted in increased vector RNA encapsidation and yielded vectors with high titre. Inefficient encapsidation by the conventionally designed vector was not due to suboptimal **Rev** responsive element (**RRE**)-**Rev** function. Varying the length of **RRE** in the vector did not change vector RNA encapsidation, nor did the introduction of a synthetic **intron** into the mutant vector. The vector RNA with the intact **splice donor** may have been excessively spliced, decreasing the amount of packageable RNA. A titre of 10(5) transducing units (TU)/ml was readily obtained for vectors with the neo or GFP transgene, and the vector could be concentrated to a titre of 1-5x10(7) TU/ml.

=>

4 ANSWER 1 OF 11 MEDLINE

AN 96078096 MEDLINE

TI Retrovirus-mediated transduction of an engineered **intron** -containing purine nucleoside phosphorylase gene.

SO HUMAN GENE THERAPY, (1995 May) 6 (5) 611-23.
Journal code: 9008950. ISSN: 1043-0342.

AU Jonsson J J; Habel D E; McIvor R S

AB We constructed and tested several **retroviral vectors** containing abbreviated purine nucleoside phosphorylase (PNP) genes in the **reverse** orientation, a strategy compatible with transduction of **intron**-containing genes. We observed two types of deletions in these vectors after one round of replication: (i) Deletions flanked by direct repeats with one copy of the repeat retained in the provirus, presumably resulting from **reverse** transcriptase slippage during (-) strand DNA synthesis. (ii) Deletions due to fortuitous splice sites in the PNP complementary strand. Two **splice donor** sites and three **splice acceptor** sites were identified in a 3.0-kb PNP minigene. We found that the **splice donor** sites (but not the **splice acceptor** sites) could be predicted by sequence analysis of the PNP complementary strand. To increase the frequency of intact PNP gene transduction, we introduced sequence modifications: The putative PNP polyadenylation signal and a truncated 117-bp 3' flank were recovered from a rearranged provirus and inserted in place of a 1.2-kb genomic 3' flank. Sequences associated with deletions were eliminated from the PNP 5' untranslated region, and two fortuitous **splice donor** signals in the complementary strand were inactivated. A **retroviral vector** LN-PMG11, containing the engineered 2.9-kb PNP minigene in the **reverse** orientation, was transduced intact in 23% (5/22) of clones after one round of replication and in 87% (20/23) of clones after a second round of replication from two primary virus producer clones. Directed mutagenesis of sequences preventing intact retroviral transduction thus provided a 2.9-kb PNP gene that was transduced intact and expressed at a high level.

(FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS, MEDICONF' ENTERED AT 17.45.50 ON 29 OCT 2002)

DEL HIS

L1 27606 S RETROVIR? VECTOR?
L2 348 S L1 AND INTRON
L3 85 S L2 AND SPLICE
L4 21 DUP REM L3 (64 DUPLICATES REMOVED)
L5 21 SORT L4 PY
L6 21 S L5 AND (DONOR OR ACCEPTOR OR SD OR SA)
L7 21 FOCUS L6 1-

=> d an ti so au ab pi l7 5 7 1-4

L7 ANSWER 5 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1998:268635 CAPLUS
DN 128:291139
TI Construction of TRIN **retroviral vectors** containing Rev-responsive element of HIV1 virus
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
IN Kingsman, Susan Mary; Kingsman, Alan John
AB **Retroviral vector** particles having an RNA genome carrying sequences which provide in the DNA provirus at least one selected gene located within an **intron** in a transcription unit of the provirus, which transcription unit further comprises a polynucleotide response element which is responsive to a nucleus to cytoplasm transport factor such as HIV Rev. These vectors have been named TRIN (Tat and Rev inducible) vectors. Expression of the selected genes is thus rendered Rev-dependent and so is dependent upon the presence of HIV. The TRIN vectors also contain the murine leukemia virus **splice donor** site, the strong CMV promoter, a packaging signal, and the HIV U5 and R regions.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9817817	A1	19980430	WO 1997-GB2859	19971017
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9747124	A1	19980515	AU 1997-47124	19971017
GB 2331989	A1	19990609	GB 1999-4143	19971017
GB 2331989	B1	20000927		
EP 931157	A1	19990728	EP 1997-909438	19971017
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001503904	T2	20010306	JP 1998-519088	19971017
US 2002141978	A1	20021003	US 1999-254529	19990804

L7 ANSWER 7 OF 21 MEDLINE
AN 2001022046 MEDLINE
TI Split-**intron retroviral vectors**: enhanced expression with improved safety.
SO JOURNAL OF VIROLOGY, (2000 Mar) 74 (5) 2365-71.
Journal code: 0113724. ISSN: 0022-538X.
AU Ismail S I; Kingsman S M; Kingsman A J; Uden M
AB The inclusion of retrovirus-derived **introns** within retroviruses-based expression vectors leads to a fraction of the resulting transcripts being spliced. Such splicing has been shown to markedly improve expression (W. J. Krall et al., Gene Ther. 3:37-46, 1996). One way to improve upon this still further might involve the use of more efficient **introns** instead of those from the provirus. Currently, however, incorporation of such **introns** remains self-defeating since they are removed in the nucleus of the producer cell. In the past, elaborate ways to overcome this problem have included the use of alphaviruses to make the vector transcripts within the cytoplasm, thus avoiding the nuclear splicing machinery during vector production (K. J. Li and H.

Garoff, Proc. Natl. Acad. Sci. USA 95:3650-3654, 1998). We now present a novel design for the inclusion of **introns** within a **retroviral vector**. In essence, this is achieved by exploiting the retroviral replication process to copy not only the U3 promoter but also a synthetic **splice donor** to the 5'-long-terminal-repeat position during reverse transcription. Once copied, synthesized transcripts then contain a **splice donor** at their 5' end capable of interacting with a consensus **splice acceptor** engineered downstream of the packaging signal. Upon transduction, we demonstrate these vectors to produce enhanced expression from near fully spliced (and thus packaging signal minus) transcripts. The unique design of these high titer and high-expression **retroviral vectors** may be of use in a number of gene therapy applications.

L7 ANSWER 1 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 2000:688391 CAPLUS
DN 133:262286
TI **Retroviral vectors** comprising functional and non-functional **splice donor** and **splice acceptor** sites
SO PCT Int. Appl., 148 pp.
CODEN: PIXXD2
IN Uden, Mark; Mitrophanous, Kyriacos
AB **Retroviral vectors** are provided comprising a functional **splice donor** site (FSDS) and a functional **splice acceptor** (FSAS) site, wherein the FSDS and the FSAS flank a first nucleotide sequence of interest (NOI) and the FSDS is upstream of the FSAS.. The **retroviral vector** is derived from a retroviral pro-vector, such that the retroviral pro-vector comprises a first nucleotide sequence (NS) capable of yielding the functional **splice donor** site (FSDS), a second NS capable of yielding the functional **splice acceptor** site (FSAS), a third NS capable of yielding a non-functional **splice donor** site (NFSDS), and a fourth NS capable of yielding a non-functional **splice** site (NFSS). The first NS is downstream of the second NS and the third NS and fourth NS are upstream of the second NS, such that after reverse transcription of the retroviral pro-vector at a desired target site the **retroviral vector** is capable of being spliced. In addn., hybrid adenolentiviral systems are provided, comprising single or multiple adenoviral primary vectors which encodes or encode a retroviral secondary vector. Murine leukemia and equine infectious anemia virus vectors (pICUT) contain a strong **splice acceptor** upstream of the **splice donor** and therefore no functional **intron**; thus, when the vector is transfected into producer cells the resulting transcripts generated will not be spliced and the packaging signal will not be lost and maximal packaging is achievable. Upon transduction, transcripts generated from integrated pICUT vectors contain a strong **splice donor** 5'- of a strong **splice acceptor**, both of which being located upstream of the neo ORF, and therefore contain a functional **intron** in the 5'UTR and thus are maximally spliced and translated. Because the **intron** is created only upon transduction it is possible to limit gene expression to either packaged or transduced cells.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000056910	A1	20000928	WO 2000-GB1091	20000322
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
GB 2362886	A1	20011205	GB 2001-20709	20000322
EP 1163356	A1	20011219	EP 2000-911135	20000322
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, SI, LT, LV, FI, RO

L7 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1999:223066 CAPLUS
DN 130:247863
TI **Retroviral vectors** comprising functional **splice donor** and **acceptor** sites formed by reverse transcription of a pro-vector
SO PCT Int. Appl., 138 pp.
CODEN: PIXXD2
IN Bebbington, Chris; Kingsman, Susan; Uden, Mark; Kingsman, Alan; Mitrophanos, Kyriacos
AB A **retroviral vector** is claimed comprising a functional **splice donor** site and a functional **splice acceptor** site; wherein the functional **splice donor** site and the functional **splice acceptor** site flank a first nucleotide sequence of interest ("NOI"); wherein the functional **splice donor** site is upstream of the functional **splice acceptor** site; wherein the **retroviral vector** is derived from a retroviral pro-vector; wherein the retroviral pro-vector comprises a first nucleotide sequence ("NS") capable of yielding the functional **splice donor** site and a second NS capable of yielding the functional **splice acceptor** site; wherein the first NS is downstream of the second NS; such that the **retroviral vector** is formed as a result of reverse transcription of the retroviral pro-vector. This system for generating **retroviral vectors** is useful for transfection and gene therapy offers improved safety.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9915683	A1	19990401	WO 1998-GB2867	19980923
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2304259	AA	19990401	CA 1998-2304259	19980923
AU 9891756	A1	19990412	AU 1998-91756	19980923
AU 750110	B2	20020711		
GB 2344592	A1	20000614	GB 2000-6992	19980923
EP 1017837	A1	20000712	EP 1998-944085	19980923
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001517452	T2	20011009	JP 2000-512972	19980923
NO 2000001486	A	20000525	NO 2000-1486	20000322

L7 ANSWER 3 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1999:223067 CAPLUS
DN 130:247864
TI **Retroviral vectors** for hematopoietic stem cell transformation comprising functional **splice donor** and **acceptor** sites formed by reverse transcription of a pro-vector
SO PCT Int. Appl., 279 pp.
CODEN: PIXXD2
IN Lewis, Claire; Binley, Katie Mary; Bebbington, Chris; Naylor, Stuart
AB A **retroviral vector** is claimed comprising a functional **splice donor** site and a functional **splice acceptor** site; wherein the functional **splice donor** site and the functional **splice acceptor** site flank a first nucleotide sequence of interest ("NOI"); wherein the functional **splice donor** site is upstream of the functional **splice acceptor** site; wherein the **retroviral vector** is derived from a retroviral pro-vector; wherein the retroviral pro-vector comprises a first nucleotide sequence ("NS") capable of yielding the functional **splice donor** site and a second NS capable of yielding the functional **splice acceptor** site; wherein the first NS is downstream

of the second NS; such that the **retroviral vector** is formed as a result of reverse transcription of the retroviral pro-vector. This system for generating **retroviral vectors** is useful for transfection and gene therapy offers improved safety. Use of this method with specific vectors for the transformation of hematopoietic stem cells is described.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9915684	A2	19990401	WO 1998-GB2885	19980923
	WO 9915684	A3	19990610		
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2303663	AA	19990401	CA 1998-2303663	19980923
	AU 9893562	A1	19990412	AU 1998-93562	19980923
	AU 747609	B2	20020516		
	GB 2345063	A1	20000628	GB 2000-6993	19980923
	GB 2345063	B2	20020724		
	EP 1017838	A2	20000712	EP 1998-946556	19980923
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2001517453	T2	20011009	JP 2000-512973	19980923
	WO 2000017371	A1	20000330	WO 1999-GB3181	19990922
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9962130	A1	20000410	AU 1999-62130	19990922
	EP 1115877	A1	20010718	EP 1999-949142	19990922
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002526083	T2	20020820	JP 2000-574270	19990922
	NO 2000001487	A	20000523	NO 2000-1487	20000322

L7 ANSWER 4 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 2000:15396 CAPLUS
DN 132:74529
TI High efficiency **retroviral vectors** that contain none of viral coding sequences
SO PCT Int. Appl., 70 pp.
CODEN: PIXXD2
IN Kim, Sunyoung; Yu, Seung Shin; Kim, Jong-mook
AB The present invention relates to improved **retroviral vectors** for gene therapy. In this invention, **retroviral vectors** with higher safety and efficiency are constructed from murine leukemia virus (MLV)-based starting vectors, MON and MIN. The improved vectors have following features: (1) sequences corresponding to MLV-derived pol gene are completely deleted in the vectors, avoiding homologous recombination which has been a baffling problem in conventional **retroviral vectors**; (2) a heterologous **intron**, splicing **acceptor** and/or non-coding sequence are/is inserted into the upstream position of cloning site, maximizing the expression of a foreign gene through efficient splicing; (3) the vectors contain either the full-length U3 sequence of 5' LTR or a strong heterologous promoter instead, permitting the abundant prodn. of RNA; (4) either IRES (internal ribosomal entry site) or internal SV40 minimal promoter is inserted into the downstream position of cloning site, enabling the simultaneous expression of two or more foreign genes. Thus, the MLV-based DONSA1 vector is constructed wherein the splicing **acceptor** of mouse Ig gene and exon 1 of human cytomegalovirus ie1 (UL123) gene are inserted

into the upstream position of the cloning site for the foreign gene; the full-length U3 sequence (-419 to -1 bp) of MLV 5' LTR is replaced with HCMV major immediate-early promoter, and SV40 minimal promoter is inserted into the downstream position of cloning site for the foreign gene. Since the improved **retroviral vectors** of this invention turn out to be safe and to express the foreign gene efficiently, they are useful for gene therapy and the like.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000000629	A1	20000106	WO 1999-KR334	19990624
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	KR 2000006334	A	20000125	KR 1999-23398	19990622
	AU 9946554	A1	20000117	AU 1999-46554	19990624
	EP 1032697	A1	20000906	EP 1999-929919	19990624
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2002519037	T2	20020702	JP 2000-557382	19990624
	US 6451595	B1	20020917	US 2000-463067	20000114

=>

(FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS, MEDICONF' ENTERED AT 17:45:50 ON 29 OCT 2002)

DEL HIS

L1 27606 S RETROVIR? VECTOR?
L2 348 S L1 AND INTRON
L3 85 S L2 AND SPLICE
L4 31 DUP REM L3 (54 DUPLICATES REMOVED)
L5 31 SORT L4 PY
L6 21 S L5 AND (DONOR OR ACCEPTOR OR SD OR SA)
L7 21 FOCUS L6 1-
L8 10 S L4 AND PY<=1996
L9 10 SORT L8 PY
L10 386 S L1 AND (SPLICE DONOR OR SPLICE ACCEPTOR OR SD OR SA)
L11 91 S L10 AND (RRE OR REV? OR TAT?)
L12 24 S L11 AND INTRON
L13 11 DUP REM L12 (13 DUPLICATES REMOVED)
L14 11 SORT L13 PY
L15 79 S L10 AND (DIFFERENT OR HYBRID OR CHIMERIC)
L16 31 DUP REM L15 (48 DUPLICATES REMOVED)
L17 31 FOCUS L16 1-
L18 13 S L17 AND PY<=1997

=> d an ti so au ab pi l18 13 7

L18 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2002 ACS
AN 1996:428590 CAPLUS
DN 125:78548
TI Modified **retroviral vectors** containing **hybrid** long terminal repeat, cytomegalovirus-IE/HIV-1-TAR/Moloney murine leukemia virus LTR, and vector use for high-level product production by recombinant technology
SO PCT Int. Appl., 125 pp.
CODEN: PIXXD2
IN Chang, Lung-Ji
AB Novel **retroviral vectors** were constructed by modifying the Moloney murine leukemia virus (MoMLV) long terminal repeat (LTR). A portion of the U3 region of the MoMLV LTR was replaced with the human cytomegalovirus immediate-early enhancer/promoter (CMV-IE) together with the human immunodeficiency virus type 1 (HIV-1) transactivation response element (TAR). Transfection studies involving the **hybrid** CMV-IE/HIV-1-TAR MoMLV LTR enhancer/promoter demonstrated that this regulatory element increases basal transcriptional levels 10- to 50-fold. Expression from the recombinant MoMLV LTR was further increased by the addn. of HIV-1 Tat. Addnl. vector modifications included the addn. of an HIV-1 extended packaging signal and 3' **splice acceptor** site. Modified **retroviral vectors** contg. the **hybrid** LTR should be useful for the prodn. of high levels of retroviral and cellular expression products.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9614332	A1	19960517	WO 1995-US14576	19951108 <--
W: AU, BB, CA, JP, KR, MX, SG, US				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5693509	A	19971202	US 1994-336132	19941108 <--
AU 9643215	A1	19960531	AU 1996-43215	19951108 <--
AU 710180	B2	19990916		
EP 791010	A1	19970827	EP 1995-942848	19951108 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 10509318	T2	19980914	JP 1995-515515	19951108

L18 ANSWER 7 OF 13 MEDLINE
AN 84251740 MEDLINE
TI Mutagenesis of the region between env and src of the SR-A strain of Rous sarcoma virus for the purpose of constructing helper-independent vectors.
SO VIROLOGY, **(1984 Jul 15)** 136 (1) 89-99.
Journal code: 0110674. ISSN: 0042-6822.
AU Hughes S; Kosik E
AB The major goal of these experiments is to derive stable, helper independent, **retroviral vectors** using the SR-A strain

of Rous sarcoma virus. Because src is flanked by direct repeats of 110 bases, both src, and sequences that replace src in vector constructions, are lost at high frequency. We have sought to eliminate this homology in order to stabilize the vectors. One copy of the direct repeat must be retained for the virus to replicate properly. Because the downstream direct repeat is linked to the polypurine tract the entire downstream direct repeat cannot easily be eliminated. We therefore sought to eliminate the upstream direct repeat. Using linkers a series of defined deletions and duplications has been created within the region between env and src. The region is relatively large, 379 bases, and has a complex history (it is derived from three **different** nucleic acid segments each with a distinct and separate origin). We show here that this region provides no functions essential for growth and, for src expression, provides only a functional **splice acceptor**. We were able to successfully replace the **splice acceptor** found in the wild type virus with an unrelated **splice acceptor** partially derived from a synthetic DNA segment. The final product is a replication competent virus that expresses src, and that lacks the entire upstream repeat. Since src is flanked by ClaI sites in these constructions, src can easily be replaced by other genes. Substituting the Tn5 neo gene for src in this construction yields a virus that expresses the neo gene nonselectively.

L Number	Hits	Search Text	DB	Time stamp
7	3	WO ADJ "9614332"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/29 18:26
15	2	wo ADJ "9915683"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/10/29 18:39
-	27	KINGSMAN-ALAN-JOHN	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/08 14:04
-	20	KINGSMAN-ALAN-JOHN and retrovir$15	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/07 10:05
-	17718	retrovir$15	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/07 10:08
-	90	retrovir$15 and (HIV WITH U3)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/07 10:09
-	58	retrovir$15 and (HIV WITH U3 WITH R)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/07 10:09
-	34	(retrovir$15 and (HIV WITH U3 WITH R)) and REV	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/07 11:14
-	1	LISZIEWICZ-JULIANNA	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/07 11:05
-	2	LISZIEWICZ-J$15	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/07 11:05
-	17718	retrovir$15	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/08 14:04
-	7505	retrovir$15 and (MULV or MLV or moMLV or murine or moloney)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2000/03/08 14:05
-	5407	(retrovir$15 and (MULV or MLV or moMLV or murine or moloney)) and (U3 or rre or rev or tat or LTR)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2002/03/08 14:06
-	2553	((retrovir$15 and (MULV or MLV or moMLV or murine or moloney)) and (U3 or rre or rev or tat or LTR)) and (donor or acceptor)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2002/03/08 14:06

-	1241	(((retrovir$15 and (MULV or MLV or moMLV or murine or moloney)) and (U3 or rre or rev or tat or LTR)) and (donor or acceptor) and HIV	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2002/03/08 14:07
-	11722	(435/320.1).CCLS.	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2002/03/08 14:08
-	421	((435/320.1).CCLS.) and ((((retrovir$15 and (MULV or MLV or moMLV or murine or moloney)) and (U3 or rre or rev or tat or LTR)) and (donor or acceptor)) and HIV)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2002/03/08 14:08
-	35	((435/320.1).CCLS.) and ((((retrovir$15 and (MULV or MLV or moMLV or murine or moloney)) and (U3 or rre or rev or tat or LTR)) and (donor or acceptor)) and HIV)) and (HIV SAME U3)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2002/03/08 14:11
-	26	((435/320.1).CCLS.) and ((((retrovir$15 and (MULV or MLV or moMLV or murine or moloney)) and (U3 or rre or rev or tat or LTR)) and (donor or acceptor)) and HIV)) and (HIV SAME U3 SAME LTR)	USPAT; US-PGPUB; EPO; JPO; DERWENT; USOCR	2002/03/08 14:11